Phytochemical screeningof*Ageratum conyzoides*L. (Asteraceae)aerial parts

ABSTRACT

Introduction: *Ageratum conyzoides* L. (Family Asteraceae) is an annual herb with a long history of traditional medicinal use in the tropical and sub-tropical region of the world. The present study was designed toscreen out the major groups of phytochemicals found in the aerial part of *A. conyzoides*.

Methodology: The aqueous and ethanolic extracts of the leaves, stem and flowers of *A. conyzoides* as well as the entire aerial part of the plant as a whole underwent qualitativeusing specific standard methods. The identified classes of metabolites in the different extracts were then quantified using spectrophotometricmethods.

Results: Phytochemical analysis of the *A. conyzoides* extracts revealed the presence of tannins, alkaloids, flavonoids, phenol, steroids, phlobatannins, saponins, and coumarins. The quantitative phytochemical analysis of the extracts of *A. conyzoides* revealed that the aqueous maceration of the leaves of *A. conyzoides* had the highest content in polyphenols, flavonoids and tannins compared to the ethanolic leaf extract as well as the ethanolic and aqueous extracts of the stems, flowers and combined aerial parts. Meanwhile, the total alkaloid content was highest in the hydroethanolic maceration of the combined aerial parts of the plant compared to the aqueous extract of the combined aerial parts as well as the aqueous and ethanolic extracts of the leaves, stems and flowers.

Conclusion: This study also shows that the extracts of *A. conyzoides* are extensively rich in secondary metabolites which could explain the application of the plant in the treatment of several ailments in traditional medicine.

Keywords:*Ageratum conyzoides*, Aerial, Phytochemical analysis, Plant metabolites, Maceration.

1. INTRODUCTION

The plant kingdom is atreasure house of potential drugs and in the recent years there has been an increasingawareness about the importance of medicinal plants[1].According to the World Health Organization more than 80% of the world's people depend on traditional medicine for their

primary healthcare needs[2].The reason being that mankind, in his attempts to find solutions to his healthcare problems,has resorted to using plant-derived therapies to remedy the shortageof reliable cures.Medicinal plants used in differentdiseases and ailments are the richest bio reservoirs of various phytochemicals[3]. Therefore, asystematic approach should be made to unlock the hidden potentialof these ethnomedicinal plants[4].

Ageratum conyzoides is one of the well-known plants which has been reported to possess diverse medicinal properties.*A. conyzoides* (Billy goat weed; Asteraceae) is native to thetropical Americas, but it is widely distributed in tropical andsub-tropical regions throughout the world[5].It has apeculiar odor likened in Australia to that of a male goat and hence its name 'goatweed'[6]. It is an annual branching herb which grows to approximately 1 m in height. The stems and leaves are covered with fine white hairs, theleaves are ovate and up to 7.5 cm long. The flowers are purple to white, less than 6mm across and arranged in close terminal inflorescences[7].The plant grows commonly in the proximity of habitation, thrives in any garden soil and is very common in waste places and on ruined sites[8].

*A. conyzoides*has been known since ancient times for its curative properties and has been utilized for thetreatment of various ailments, such as burns and wounds, headaches pneumonia, analgesic, inflammation, asthma,spasmodic and hemostatic effects, stomach ailments, gynecological diseases, leprosy and other skin diseases[9]. In Cameroon and Congo, it is used to treatfever, rheumatism, headache and colic[10]. The aqueous extract of leaves or wholeplant is utilized to treat colic, cold and fevers, rheumatism in Brazil [11]. Due to the fact that different parts of *A. conyzoides* have traditional medicinal applications, the present study was therefore planned to define and quantify the phytochemicals present in the aerial parts(leaves, flowers, stem) of the plant in order to provide a scientific basis to justify its traditional therapeutic application.

Taxonomic classification

Figure 1: Photo of *A. conyzoides*. (From this study)

Ageratum conyzoides belongs to: [12]

Kingdom: Plantae

Subkingdom: Viridiplantae

Division: Tracheophyta

Class: Magnoliopsida

Superorder: Asteranae

Order: Asterales

Family: Asteraceae

Genus: Ageratum L.

Species:*Ageratum conyzoides* L.

MATERIALS AND METHODS

Sample collection and authentication

The plant specimen was deposited and identified in the National Herbarium of Cameroon (NHC) by comparing with reference specimen number 61801/NHC. Fresh mature plants were harvested in Yaoundé in January 2023. The harvested plant was separated into individual aerial parts (leaves, stems and flowers)and aerial part as a whole of *A. conyzoides.* The plant materialwas air-dried separately away from sunlight at room temperature under shade and ground to fine powder using a milling machine. The fine powder was stored in clean labeled airtight containers till required for analysis.

Preparation of plant extracts

In this study, aqueous and hydroethanolic extracts of each aerial part (leaves, stem and flowers) as well as the whole aerial part of *A. Conyzoides*were prepared separately using the maceration technique.

Maceration

In maceration, whole or coarsely powdered plant is kept in contact with the solvent in a stoppered container for at least two days with frequent agitation until soluble matter is dissolved. At the end of extraction, the micelle is separated from marc by filtration and from the menstruum by evaporation in an oven. [13]

Procedure

In this process, 100 g of coarsely powdered plant's aerial part, leaves, stem and flowers were placed in a stoppered container with 1000 mL of solvent (distilled water, hydro-ethanolic solution 50:50) and allowed to stand at room temperature for a period of 72 hours with frequent agitation until the soluble matter has dissolved. The mixture was then strained, the marc (the damp solid material) pressed, and the combined liquids were clarified by filtration using Whatman paper. At the end of extraction, the micelle was separated from marc by filtration and

placed in an oven at 50°C[14]. The dried extract obtained was stored in an air-tight container and kept in refrigerator at 4ºC for their future use in phytochemical analysis.

Determination of extraction yield (%)

Each extract of *A. conyzoides* was weighed in order to determine the percentage yield obtained from the initial powder quantity.The yield of evaporated dried extracts based on dry weight basis was calculated from the equation:

$$Percentage\ yield\ (\%) = \frac{mass\ of\ the\ extract\ obtained}{mass\ of\ the\ initial\ powder} \times 100$$

Phytochemical screening

Qualitative analysis

The various extracts of the leaves, stem and flowers of *A. conyzoides* as well as the aerial part as a whole were screened qualitatively for the phytochemical constituents utilizing standard methods of analysis by Balamurugan (2019) and Mahendru et al. (2024)[15,16]. The filtrates of the *A. conyzoides* extracts were analyzed for the following phytochemicals: carbohydrates, proteins, glycosides, tannins, alkaloids, flavonoids, saponins, resins, quinones, cardiac glycosides, coumarins, steroids, phenols, anthraquinones and phlobatannins. Thetests were based on the visual observation of color changeor formation of a precipitate after the addition of specificreagents.

Qualitative analysis of primary metabolites

Test for carbohydrates

Fehling's test: About 1 mL of the filtrate was taken to which 1 mL of Fehling's reagent was added and boiled in a water bath. The appearance of red precipitate indicated the presence of sugars.

Molisch's test: To about 2mL of the sample, 2 drops of alcoholic solution of α-naphthol were added to the mixture after being properly shaken. Few drops of conc. H_2SO_4 was added along the sides of the test tube. A violet ring indicated the presence of sugars. [16]

Test for proteins

Biuret test: To 2 mL of filtrate was added 1 drop of 2% copper sulphate solution; and 1 mL of 95% ethanol. This was followed by excess addition of KOH. The appearance of pink color indicated the presence of protein.

Millon's test: To 2 mL of filtrate was added a few drops of Millon's reagent. The appearance of a white precipitate indicated the presence of proteins. [15]

Test for lipids

To the extract were added a few drops of 0.5N alcoholic KOH and few drops of phenolphthalein. This mixture was heated for about 2 hours. The formation of soap or partial neutralization of alkali indicated the presence of fixed oils or fats. [15]

Miscellaneous compounds

Test for resins

1 mL of extract was taken and to this few mL of acetic anhydride was added to 1 mL of conc. H_2SO_4. The appearance of orange to yellow color indicated the presence of resins. [15]

Test for gums and mucilage

To 1 mL of extract, distilled water, 2 mL of absolute ethanol was added accompanied by constant stirring. The appearance of a white or cloudy precipitate indicated the presence of gums or mucilage.[15]

Vitamin C

In 5ml of distilled water, 1ml of the sample was diluted and a drop of 5% sodium nitroprusside and 2ml of NaOH wasincluded in the mixture. Few drops of HCl were incorporated dropwise, the yellow color turns blue. This indicated the presence of vitamin- C.[15]

Carotenoids

In two test tubes containing 2 mL of the plant extract and 2 mL of water each, was incorporated 2 mL of HCL and 2 mL of H_2SO_4. The appearance of a blue-green color in both test tubes confirmed the presence of carotenoids.[15]

Qualitative analysis of secondary metabolites

Test for anthraquinones

To 5ml of extract, few ml of conc.H_2SO_4 was added and 1ml of diluted ammonia was equally added to it. The appearance of rose pink confirmed the presence of anthraquinones.[15]

Test for quinones

To 1 mL of extractwas addedalcoholic KOH. The presence of red to blue color indicated the presence of quinones.[15]

Test for alkaloids

Mayer's test: to a few mL of filtrate, 2 drops Mayer's reagent was added. A creamy or white precipitate showed a positive result for alkaloids.

Wagner's test: (iodine – potassium iodine reagent): To about an mL of extract few drops of Wagner's reagent was added. Reddish – brown precipitate indicated presence of alkaloids.

Hager's test: To a few mL of the filtrate was added 2 mL of Hager's reagent. A creamy white/ yellow precipitate showed a positive result for alkaloids.

Tannic acid test: To the acidified extract, 10% tannic acid solution was added. A buff color precipitate indicated the presence of alkaloids. [15]

Test for glycosides

2 mL of extract was mixed with a few drops of glacial acetic acid containing traces of ferric chloride and 0.5 mL of conc. H_2SO_4. The production of blue color was positive for glycosides. [15]

Test for cardiac glycosides (Keller-Killani test)

5mL of solvent extract was mixed with 2mL of glacial acetic acid and a drop of ferric chloride solution followed by the addition of 1mL of conc. H_2SO_4. A brown ring in the interface indicated the presence of deoxy sugars of cardenolides. The appearance of a violet ring beneath the

brown ring or the formation of an acetic acid layer in the form of a green ring indicated the presence of cardiac glycosides.[15]

Test for polyphenols

To the extract was added few drops of 5% ferric chloride. The formation of dark green color indicated presence of polyphenols.To 1 mL of extract, few drops of 5% solution of lead acetate were added. The appearance of yellow precipitate was considered positive for polyphenols.[15]

Test for tannins

To 5mL of extract, few drops of neutral 5% ferric chloride solution was added. The production of dark green color indicated the presence of tannins.[16]

Differentiation of catechic and gallic tannins

It is obtained by STIASNY reaction, which is carried out in the following manner. To 30mL of the extract, we add 15mL of STIASNY reagent (10mL of 40 % formalin and 5 mL of concentrated HCl) and heated for 15 minutes in a water bath at 90 °C.

Catechic tannins: The obtention of a precipitate indicated their presence;
Gallic Tannins: After filtration, the filtrate was saturated with powdered sodium acetate, then 1 mL of a solution of 1 % ferric perchloride ($FeCl_3$). The presence of gallic tannins not precipitated by the STIASNY reagent was indicated by the development of a shade dark blue. [16]

Test for flavonoids

In a test tube, 2 mL of the 1 % extract was added. Add 1 mL of sodium hydroxide (NaOH) of 2N. Theformation of a yellow-orange coloration indicated the presence of flavonoids. The addition of afew drops of sulfuric acid and the color change observed confirmed the presence of flavonoids.[16]

Test for phlobatannins

Aqueous extract was boiled with diluted HCl. The deposition of reddish precipitate indicated the presence of phlobatannins. [15]

Test for beta cyanide

2 mL of the extract was introduced in a test tube and 2 mL of 2N NaOH was added. The mixture was placed in a boiling water bath for 5 minutes. The appearance of a yellow coloration indicated the presence of betacyanin.[16]

Test for saponins

0.5 mg of extract was vigorously shaken with few mL of distilled water. The formation of frothing was considered positive for saponins. The froth from the above reaction was taken and few drops of olive oil were added and shaken vigorously. The formation of emulsion was observed. [16]

Test for steroids

2 mL of extract with 2 mL of chloroform and 2mL of concentrated H_2SO_4 was added, the appearance of red color and yellowish green fluorescence indicated the presence of steroids. [15]

Test for anthocyanins

2 mL of aqueous extract was taken to which was added 2N HCl followed by the addition of ammonia. The conversion of pink-red colour to blue-violet indicated the presence of anthocyanins.[15]

Test for coumarins

To 2 mL of the extract, 3 mL of 10% aqueous solution of NaOH was added. The production of yellow color indicated the presence of coumarins. [15]

Test for oxalates

In a test tube was incorporated 2 mL of the extract. A few drops of ethanoic acid were added. The obtentionof a greenish-black color indicated the presence of oxalates.[16]

Test for chalcones

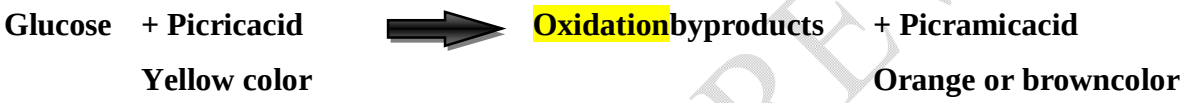
To 5 mL of the plant extract was added 2 mL of ammonia solution. The appearance of a red color confirmed the presence of chalcones. [16]

QUANTITATIVE ANALYSIS

Depending on the qualitative results, the quantitave assay was carried out for carbohydrates, proteins, polyphenols, alkaloids and flavonoids.

Determination of total soluble carbohydrates

Picric acid is reduced into picramic acid by glucose. The intensity of the orange color of picramic acid will be proportional to the concentration of glucose. The orange color of picramic acid produced, whose maximum absorption is around 570 nm, is proportional to the quantity of phenolic compounds present in the plant extract[17]. The reaction is as follows:



In a test tube, 1000 µL of 13% picric acid and 1000 µL of 4% sodium hydroxide were added to 1000 µL of the extract at a concentration of 1000 µg/mL. The tube was placed in a boiling water bath for 10 minutes. The calibration curve was used during the reading of the optical density at 570 nm against the extract and blank concentration. The amount of carbohydrate content was expressed as equivalent of glucose per mg of dry extract of the plant (EG/g)[17].

Determination of total protein content

Lowry's method is another colorimetric protein assay method, complementary to that of Biuret. In fact, the protein first reacts with an alkaline cupric reagent (Gornall's reagent of the biuret method) then a second reagent, called phosphotungstomolybdic (Folin-Ciocalteu's reagent), is added. This reagent allows the reduction of aromatic amino acids (tyrosine and tryptophan) leading to the formation of a dark blue colored complex whose absorbance is measured between 650 and 750 nm[18].

BSA was used as standard reagent for preparing the standard curve against which the unknown concentration of proteins was estimated. In a test tube, 1000 µL of alkaline reagent was added to 1000 µL of the extract at 1000 µg/mL and incubated for 10 minutes followed by the addition of 500 µL of the Folin-Ciocalteu reagent (diluted to 1/10th) was followed immediately by

incubation at room temperature for 20 minutes in darkness to allow for the development of the blue color. The absorbance was read at 760 nm on a spectrophotometer. The quantity of protein content after that the absorbance was measured at 760 nm and the amount of protein expressed as µg/mL of BSA (EBSA/g)[18].

Evaluation of total phenolic contents

To determine the total phenolic content, we used the method of Folin-Ciocalteu. Folin-Ciocalteu reagent is a yellow-colored acid consisting of a mixture of two acids: phosphotungstic acid ($H_3PW_{12}O_{40}$) and phosphomolybdic acid ($H_3PMo_{12}O_{40}$). It is reduced during the oxidation of polyphenols to form a stable blue complex of tungsten and molybdenum oxides. The color produced, whose maximum absorption is around 760 nm, is proportional to the quantity of phenolic compounds present in the plant extract.[19]

1000 µL of the Folin-Ciocalteu reagent (diluted to 1/10th) was added to 1000 µL of the extract at 1000 µg/mL and the whole was incubated for two minutes at room temperature. Then, 1000 µL of sodium bicarbonate at 75 g/L was added, followed immediately by incubation at room temperature for 90 minutes. The absorbance was read at 760 nm on a spectrophotometer. The quantity of phenolic compounds, expressed in milligrams of gallic acid equivalent per gram of dry weight of the plant (mg GAE/g), was determined by the calibration curve varying from 0 to 1000 mg/mL, made with different concentrations of gallic acid.

Evaluation of total flavonoid contents

The quantification of flavonoids was carried out using aluminum trichloride and sodium hydroxide. The aluminum trichloride forms a yellow complex with flavonoids and the soda forms a pink colored complex which absorbs in the visible electromagnetic spectrum at 510 nm[20].

In each tube, we introduced 1000 µL of the extract at 1000 µg/mL, followed by the addition of 150 µL of sodium nitrite ($NaNO_2$) at 5%. After 5 minutes, 150 µL of aluminum trichloride ($AlCl_3$) at 10% (m/v) was added to the mixture. After 6 minutes of incubation at room temperature, 500 µL of 4% sodium hydroxide (NaOH) was added. The mixture was stirred immediately to homogenize its content. The absorbance of the pinkish colored solution was read at 510 nm against a blank. A calibration curve was produced in parallel under the same operating conditions using quercetin as a positive control. The total flavonoid content of the plant extracts

studied was expressed in milligram equivalent of quercetin per gram of dry plant matter (mg EQ/g). [21]

Evaluation of total flavonols content

The sample containing flavonols results in the formation of a green color when reacted with aluminum chloride and sodium acetate, and the samples are read at 440 nm in a UV-Vis spectrophotometer.[21]

In a 10 ml test tube, 1000µl of extracts at a dose of 1000µg/ml, and 1000µl of $AlCl_3.6H_2O$ 2%, 600µl of sodium acetate 50g/l, was completed with distilled water to the volume of 3000µl. The tubes were incubated at room temperature for 2.5 hours. The solution was mixed well and the absorbance was measured against the blank not containing the extract at 440 nm. The standard curve for flavonols was carried out using a standard solution of quercetin (0 to 200 µg/l) according to the same procedure as that which was previously described. The total flavonols were expressed in milligrams of quercetin equivalents per g of dried fraction (mg EQ/g). [21]

Evaluation of total alkaloids content

The alkaloid, in contact with concentrated sulfuric acid and potassium dichromate, develops a violet line which turns blue then green, so the maximum absorption proportional to the intensity of the color developed is 650 nm[22].

In a glass test tube, to 1000µl of the sample at 1000µg/ml, was added 1000µL of 5% potassium dichromate and incubation was allowed for 5min at 30°C. 8000µl of concentrated sulfuric acid was then added to the tubes content and mixed. The tubes at room temperature were allowed for 20min to observe any color change and then read at the optical density of 650nm against the blank or the sample. The alkaloid concentration was obtained from the calibration curve and expressed in milligrams of quinine hydrochloride equivalents per gram of dried fraction (mg QHE/g)[22].

Evaluation of total tannins content

The technique for assaying condensed tannins by the Folin-Ciocalteu method is based on the reduction of phosphomolybdic and tungstic acid in an alkaline medium, in the presence of tannins, to give a blue color whose intensity is measured between 640 and 760 nm [23].

In a 10 ml test tube, 1000µl of extracts at a dose of 1000µg/ml, 200µl of Folin's reagent diluted to one tenth and 1000µl of 35% Na_2C0_3 were added. The solution was mixed well and the absorbance was measured against the blank containing no extract at 700 nm. The standard curve for total tannins was produced using a standard solution of tannic acid (0 to 500 µg/ml) according to the same procedure as that described previously. Total tannins were expressed in milligrams of tannic acid equivalents per g of dried fraction (mg TAE/g). [23]

Ethical considerations

The study was conducted after having approval from the institutional review board of the Faculty of Medicine and Biomedical Sciences. An authorization was obtained from the head of animal house affiliated with the laboratory of pharmaco-toxicology and pharmacokinetics.

RESULTS

EXTRACTION YIELD OF EXTRACTS

The extraction yield of each extract of *A. conyzoides* is presented in table 1. The extraction yield of the aerial part of *A. conyzoides* was the lowest among the aqueous and hydroethanolic extracts of the plant. While the flower hydroethanolic extract had the highest yield among the hydroethanolic extracts of the plant, the leaf aqueous extract had the highest yield among the plant's aqueous extracts.

Table 1: Extraction yield of the extracts of *A. conyzoides.*

Solvent	**Aqueous maceration**				**Hydroethanolic maceration**			
	AP of plant	**Leaf**	**Stem**	**Flower**	**AP of plant**	**Leaf**	**Stem**	**Flower**
Extraction yield (%)	9.2	13.1	10.8	11.3	8.3	10.3	13.4	16.3
Texture	viscous	viscous	powder	powder	viscous	viscous	viscous	viscous

Legend: AP = Aerial part

PHYTOCHEMICAL ANALYSIS

Evaluation of the primary metabolites and vitamins

Analysis of *A. conyzoides* extracts revealed the presence of carbohydrates, total proteins, vitamin C and the absence of total lipids and carotenoids in both its aqueous and hydroethanolic extracts as shown in table 2.

Table 2: Qualitative analysis of primary metabolites in *A. conyzoides* extracts.

Metabolites	Test	Aqueousmaceration				Hydroethanolicmaceration			
		AP of plant	Leaf	Stem	Flower	AP of plant	Leaf	Stem	Flower
Vitamin C	DNPH	+	+	+	+	+	+	+	+
Carotenoids		-	-	-	-	-	-	-	-
Carbohydrates	Molisch test	+	+++	+	++	++	+	++	++
	Fehling's test	+	+++	+	++	++	+	++	++
Proteins	Biuret test	++	+	+	+	++	+	+	+
	Millon's test	++	+	+	+	++	+	+	+
Lipids	Ethanol/H_2O	-	-	-	-	-	-	-	-
	NaOH/Ethanol	-	-	-	-	-	-	-	-

Key: – = negative, + = positive,AP= Aerial part

Qualitative analysis of secondary metabolites

Preliminary phytochemical screening of the aqueous and ethanolic extracts of the aerial part of the plant and its individual parts (leaf, flower and stem) revealed the presence of various bioactive components which include polyphenols, alkaloids, flavonoids, coumarins and tannins though there were some inconsistencies with regard to a few metabolites as shown in Table 3. Saponins and mucilages were found to be present in all the plant extracts except in the aqueous maceration of the aerial part of the plant and stem hydroethanolic extract. Steroids and cardiac glycosides were present only in the aqueous and hydroethanolic extracts of *A. conyzoides* leaves.

Table 3: Qualitative analysis of secondary metabolites of *A. conyzoides* extracts.

Metabolite	Reagents	Aqueousmaceration				Hydroethanolicmaceration			
		AP of plant	Leaf	Stem	Flower	AP of plant	Leaf	Stem	Flower
Polyphenols	$FeCL_3$	+	+++	+	+	+	+	+	+
	Lead acetate	+	+++	+	+	+	+	+	+
Alkaloids	Wagner	++	++	+	+	+++	+++	++	++
	Hager	++	++	+	+	+++	+++	++	++
	Valse-Mayer	++	++	+	+	+++	+++	++	++
	Tannicacid	++	++	+	+	+++	+++	++	++
	$AlCL_3$	+	+++	+	+	+	+	+	+

Flavonoids	$NaOH/H_2SO_4$	+	+++	+	+	+	+	+	+
	Catechic tannins	+	+++	+	+	+	+	+	+
Tannins	Gallic tannins	+	+++	+	+	+	+	+	+
Saponins	Distilled water	-	+	+	+	+	+	-	+
Mucilage	Absoluteethanol	-	+	+	+	+	+	-	+
Steroids	Acetic anhydride	-	+	-	-	-	+	-	-
Coumarines	HNO_3	+	+	+	+	+	+	+	+
Oxalate	Ethanoicacid	-	-	-	-	-	-	-	-
Quinones	Sulfuricacid	-	-	-	-	-	-	-	-
Betacyane	NaOH	+	+	+	+	+	+	+	+
Phlobotannins	HCl	+	+	+	+	+	+	+	+
Anthocyane	NH_4OH	-	-	-	-	-	-	-	-
Cardiac glycosides	Glacial acetic	-	-	-	-	-	-	-	-
Resins	Anhydrousacetic	-	-	-	-	-	-	-	-

Key: – = absent, + =present, AP= aerial part

3.3.Quantitative analysis of plant metabolites

The various extracts of the plant contained carbohydrates, proteins, tannins, flavonoids, flavonols and alkaloids which were then quantified using a UV spectrophotometer. Their concentrations are shown in table 4.

Table 4: Concentration of metabolites in *A. conyzoides* extracts.

Metabolites	**AP of Plant**		**Leaf**		**Stem**		**Flower**	
	AQ	**HET**	**AQ**	**HET**	**AQ**	**HET**	**AQ**	**ET**
Carbohydrates	85,57 ±	92,45 ±	157,77 ±	59,20 ±	34,68 ±	80,06 ±	82,63 ±	74,46 ±
(mgEG/g)	$2,52^{f,i}$	2,90	4,25	$2,36^{c,i,l}$	$2,08^{f,l}$	$1,25^{c}$	$1,95^{l}$	$1,96^{c,l}$
Total proteins	561,79 ±	767,18 ±	1059,23 ±	530,26 ±	343,85 ±	681,79 ±	541,79 ±	521,03 ±
(µg BSA/g)	$107,30^{f,i}$	26,89	4,68	$25,68^{c,i,l}$	$6,30^{f,l}$	$28,91^{c}$	$8,44^{f}$	$23,09^{c,l}$
Totalpolyphenols	430,27 ±	364,93 ±	633,87 ±	356,00 ±	288,13 ±	366,67 ±	422,27 ±	408,80 ±
(mg GAE/g)	$14,83^{f,i}$	$5,66^{l}$	5,60	$3,12^{l}$	$17,22^{f,l}$	$7,49^{l}$	$3,78^{f}$	7,11
Total flavonoids	407,71 ±	425,35 ±	524,37 ±	361,73 ±	181,73 ±	311,43 ±	388,29 ±	362,02 ±
(mg EQ/g)	$16,29^{f}$	3,07	15,63	$10,37^{c}$	$19,28^{c,f,l}$	$7,96^{c,f,l}$	$14,64^{f}$	$4,87^{c}$
Total flavonols	637,14 ±	590,36 ±	779,76 ±	435,95 ±	261,67 ±	255,24 ±	755,71 ±	451,79 ±

(mg EQ/g)	14,38[f]	3,44	19,12	7,41	7,90[c,f,l]	22,38	24,77	3,11
Total tannins (mgTAE/g)	170,56 ± 5,70[f]	128,93 ± 1,65	352,81 ± 2,82	117,66 ± 2,34	153,35 ± 5,30[a,f,k]	131,53 ± 7,00[a,g,l]	171,72 ± 5,74[f]	139,78 ± 3,93
Total alkaloids (mg QHE/g)	1090,56 ± 34,05	1891,67 ± 35,28	1152,78 ± 65,18	1535,00 ± 131,70[b]	506,11 ± 39,77[c,f,l]	1483,89 ± 30,25[c,e]	899,44 ± 11,71[b,c]	1162,78 ± 35,64[c,e,k]

Key: AP = Aerial Part, AQ = aqueous, HET = hydro ethanol

The results are expressed as mean ± SEM with n = 3; Data analysis was performed using the ANOVA test, followed by Turkey Kramer post hoc multiple comparison test. The differences were considered significant from *a < 0,05*; *b < 0,01*; *c < 0,001* with the aerial part of the plant, *d <0,05*; *e < 0,01*; *f < 0,001* for the leaf; *g < 0,05*; *h < 0,01*; *i < 0,001* for the stem; *i < 0,05*; *j < 0,01*; *k < 0,001* for the flower

Total carbohydrate content

The distribution of total carbohydrates in the different plant extracts shows that in the aqueous extracts, the leaves contain the highest concentration in total carbohydrates with a significant difference compared to the other parts of the plant as well as the aerial part of the plant as a whole. with a *p-value < 0. 001*. In the hydro-ethanolic extracts, we note the preponderance of the aerial part of the plant compared to the other parts which are the leaves with a significant difference with a *p-value* < 0.001. Among the aqueous extracts of *A. conyzoides*, the stem has the lowest content in carbohydrates meanwhile among the hydroethanolic extracts of the plant, the flowers have the lowest content in soluble carbohydrates.

Total protein content

The distribution of total proteins in the different extracts of the plant shows that in the aqueous extracts obtained by maceration, the leaves present the highest concentration in comparison with the entire aerial part of the plant, the flowers and the stems with *a p-value < 0.001*. We note however in the hydro-ethanolic extracts, a higher concentration of proteins in the aerial part of plant in comparison with the leaves, stems and flowers. The total protein content in the flower ethanolic extract is low as compared to the other ethanolic extracts of the plant while the stem aqueous extract is the lowest in comparison to the other aqueous extracts of the plant.

Total polyphenols content

The distribution of total polyphenols in the different extracts of the plant shows that in the aqueous extracts obtained by maceration, the leaves presented the highest concentration in comparison with the aerial part of the plant, flower and stem with a *p-value < 0. 001*. However, we note that in the hydro-ethanolic extracts, their concentration was highest in the flowers as compared to the leaf, stem and the aerial part of the plant with a *p-value of 0.001*. The stem aqueous extract has a significantly low content in polyphenols amongst the aqueous *A. conyzoides* extracts while the leaf of *A. conyzoides* ethanolic extract has the lowest concentration in polyphenols as compared to other ethanolic extracts of the plant.

Total flavonoid content

The distribution of total flavonoids in the different extracts of the plant shows that in the aqueous extracts obtained by maceration, the leaf presents the highest concentration in comparison with the whole aerial part of the plant, flower and stem with a p-value < 0.001. Meanwhile, we notice that in the hydro-ethanolic extracts, total proteins are highest in the aerial part of the plant in comparison with the leaves, stems and flowers with a p-value of < 0.001. Generally, both the aqueous and ethanolic extracts of the stem of *A. conyzoides* presents the lowest content in flavonoids as compared to the leaves, flowers and aerial part of the plant's aqueous and ethanolic extracts respectively.

Total flavonols content

The distribution of total flavonols in the different extracts of the plant shows that in the aqueous extracts obtained by maceration, the leaves present the highest concentration in comparison with the whole aerial part of the plant, the flowers and stem with a p-value < 0.001 whereas, in the hydro-ethanolic extracts, we observe that the aerial part of the whole plant has the highest concentration in flavonols in comparison with the leaves, stems and flowers with a p-value of < 0.001. We also notice that the stem aqueous and ethanolic extracts have the lowest in flavonols in comparison to the aqueous and ethanolic extracts of the leaves, flowers and aerial part as a whole of *A. conyzoides* respectively.

Total tannins content

The distribution of total tannins in the different extracts of the plant shows that in the aqueous extracts obtained by maceration, the leaves present the highest concentration in comparison with

the entire aerial part of the plant, flowers and stems with a *p-value* < *0.001* whereas, we note in the hydro-ethanolic extracts, a slightly higher concentration in the flowers in comparison to the leaves, stems and the aerial part of the plant. We equally note that total tannins content among the aqueous extracts of *A. conyzoides* is lowest in the stem meanwhile among the ethanolic extracts of the plant, it is lowest in the leaves.

Total alkaloid content

The distribution of total alkaloids in the different extracts of the plant shows that in the aqueous extracts obtained by maceration, the leaves present the highest concentration in comparison to the entire aerial part of the plant, flowers and stems with a p-value < 0.001. However, in the hydro-ethanolic extracts, the aerial part of the plant in comparison to the leaves, stems and flowers had the highest concentration in alkaloids. Also, we note that, the total alkaloid content among the aqueous extracts of *A. conyzoides* is lowest in the stem meanwhile among the ethanolic extracts of the plant, it is lowest in the flowers.

DISCUSSION

Phytochemicals are naturally occurring substances found in plants which provides health benefits.These are known as secondary metabolites and may often be created by modifiedsynthetic pathways from primary metabolite or share substrates of primary metabolite origin[24]. *Ageratum conyzoides* L., Asteraceae, is an annual herbaceous plant with a long history of traditionalmedicinal uses in several countries of the world. Earlier studies conducted by researchers such as Fatima *et al.* (2016), Odeleye *et al.* (2014) and Onerijiofi and Isola (2019) on *A. conyzoides*[1,25,26] had indicated the presence of phytochemicals in the *A. conyzoides*. The minor differences observed between the phytochemicals detected in this study and those of earlier authors could have arisen from differences in extraction methods, nature, concentration and polarity of solvent used, environmental factors and time of harvest [27].

This study revealed the presence of phytochemicals in all aerial parts of *A. conyzoides* such as polyphenols, alkaloids, flavonoids, coumarins and tannins. Most of these phytochemicals are known to possess pharmacological applications.For instance, flavonoids are a large class of natural compounds that are widely found in the plant kingdom found to possess a diverse range

of bioactivities, such as antioxidation, antihyperlipidemic, antifatigue, antiaging, and atherosclerosis-prevention activities[28,29]. Meanwhile, alkaloids possess antimicrobial activity which could explain why the plant is used for the traditional treatment of infectious diseases [30]. In addition, saponins, tannins and terpenoids have been reported to have dramaticphysiological antibacterial, antifungal and antioxidant properties [31]. In all, this study shows that the extracts of *A. conyzoides* are extensively rich in secondary metabolites thereby justifying its use in the treatment of several ailments by traditional practitioners.

The quantitative phytochemical analysis of the extracts of *A. conyzoides* revealed that the aqueous maceration of the leaves of *A. conyzoides* had the highest content in most of the metabolites. It contained 157,77 ± 4,25 mg EG/mg of total carbohydrate content, 1059,23 ± 4,68 µg BSA of totalprotein content, 633,87 ± 5,60 mg GAE/g of total phenolic content, 524,37 ± 15,63 mg EQ/g of total flavonoid content, 779,76 ± 19,12 mg EQ/g of total flavonols content and 352,81 ± 2,82 mg TAE/gm of total tannins content. However, an exception was observed with the total alkaloid content which was highest in the hydroethanolic maceration of the aerial part of the plant at a concentration of 1891,67 ± 35,28 mg QHE/g. In this study, the total phenolic content in the aqueous and ethanolic maceration of the leaves was similar to what was obtained in a study conducted by Pawan Kumar *et al.* (2012) [32]on the acetone and hexane extracts of the leaves of *A. conyzoides* and higher than what was found by Puro *et al.* (2018) [33]in the methanolic leaf extract of the plant. However, the amount of total flavonoid content in the aqueous and ethanolic extracts of the leaves in this study was much lower than what was obtained by Pawan Kumar *et al.* 2012 [32]. The differences are could be related to the difference in the extraction solvents.The high content in antioxidant phytochemicals in the leaf extracts of the plantcould explain its widespread use in the treatment of several oxidative stress related ailments such as cancer, erectile dysfunction, rheumatoid arthritis, kidney and liver diseases[34,35] . Also, due to the fact that the leaf extracts contained the highest contents in most secondary metabolites, this could explain why it is the most commonly applied part of *A. conyzoides*.

CONCLUSION

In conclusion, the present study shows that *A. conyzoides* aqueous and hydro-ethanolic maceration extractsof the leaves, stem and flowers as well as the aerial part as a whole contain

polyphenols, saponins, alkaloids, flavonoids, catechic tannins, gallic tannins, coumarins, quinones and phlobatannins. The present work also shows that mostprimary and secondary metabolites are predominantly found in aqueous maceration of the leaves of *A. conyzoides*as compared to the aqueous and ethanolic extracts of all other parts of the plant as well as its aerial part as a whole.This study couldserve as a benchmark for further pharmacological studieson the plant that may be harnessed for drugdevelopment in the future.

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